The use of quaternary pyridinium salts as vasoprotective agents

The present invention relates to the use of certain quaternary pyridinium salts for the preparation of a vasoprotective agent for the treatment and/or prevention of conditions or diseases associated with dysfunction of vascular endothelium, oxidative stress, and/or insufficient production of endothelial prostacyclin (PGI_2), as well as the use of pyridinium salts for oral use in diet supplementation.

There is increasing evidence that endothelial dysfunction plays a key role in the formation and progression of atherosclerotic plaque. In the light of the recently gained knowledge endothelial dysfunction has diagnostic, prognostic and therapeutic significance (Heitzer T, Schlinzig T, Krohn K, Meinertz T, Munzel T. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. *Circulation* 2001;104:2673-2678; Schachinger V, Britten MB, Zeiher AM. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. *Circulation* 2000;101:1899-1906; Perticone F, Ceravolo R, Pujia A, Ventura G, Iacopino S, Scozzafava A, Ferraro A, Chello M, Mastroroberto P, Verdecchia P, Schillaci G. Prognostic significance of endothelial dysfunction in hypertensive patients. *Circulation* 2001;104:191-196; Suwaidi JA, Hamasaki S, Higano ST, Nishimura RA, Holmes DR, Jr., Lerman A. Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. *Circulation* 2000;101:948-954). Clinically, endothelial dysfunction is identified as impairement of biological activity of NO, which is diagnosed as an impairment of vasodilating NO activity. Impairment of biological NO activity coincides with oxidative stress (Heitzer T, Schlinzig T, Krohn K, Meinertz T, Munzel T. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. *Circulation* 2001;104:2673-2678) and impairment of PGI_2 synthesis in endothelium (Kyrle PA, Minar E, Brenner B, Eichler HG, Heistinger M, Marosi L, Lechner K. Thromboxane A_2 and prostacyclin generation in the microvasculature of patients with atherosclerosis - effect of low-dose aspirin. *Thromb Haemost* 1989;61:374-377), although level of PGI_2 may be elevated. Indeed, several years ago it was proposed that lipid peroxidation might promote development of atherosclerosis owing to selective impairment of prostacyclin synthesis in endothelial

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cells and subsequent activation of platelets (Gryglewski RJ. Prostacyclin and atherosclerosis. *TIPS* 1980;1:164-168; Gryglewski RJ. Prostaglandins, platelets, and atherosclerosis. *CRC Crit Rev Biochem* 1980;7:291-338; Gryglewski RJ, Szczeklik A. Prostacyclin and atherosclerosis - experimental and clinical approach. 1983;213-226). This hypothesis was then supported experimentally. There are many evidences now that impairment of PGI_2 synthesis in endothelium may lead to the excessive stimulation of TP receptors in endothelium and vascular smooth muscle cells by TXA_2, PGH_2 or other eicosanoids. These mechanisms lead to excessive vasoconstriction, platelet aggregation, and inflammatory response of endothelium as well as endothelial apoptosis. This means that impairment of endothelial synthesis of PGI_2 may trigger or enhance inflammatory and thrombotic processes in vascular wall, which are now considered to be the key elements in the development of atherosclerosis (*atherothrombosis*). Due to the above, one can believe that the ability of certain quaternary pyridinium salts to stimulate PGI_2 production in endothelium can bring anti-atherosclerotic effects. Similarly, in many other diseases (mentioned above) wherein endothelial dysfunction plays a role in pathogenesis, pharmacological enhancement of endothelial PGI_2 production by theses salts can bring therapeutic effects. In summary, we believe that these salts have therapeutic potential in any diseases where dysfunction of vascular endothelium, oxidative stress, and/or insufficient production of endothelial prostacyclin (PGI_2) play a role.

In the publication of the international patent application no. WO00/40559 therapeutic and cosmetic uses of certain nicotinamide derivatives, 1,3-disubstituted pyridinium salts, including 1-methylnicotinamide (MNA^+) and 1-methyl-N'-(hydroxymethyl)-nicotinamide ($MNAF^+$) salts were disclosed. It was reported that these derivatives have the utility in topical treatment of skin diseases, in particular crural ulceration, acne, psoriasis, atopic dermatitis, vitiligo, as well as burns and scalds and in wound healing. These compounds have also the activity of promoting hair re-growth, therefore they are useful in the treatment of hair loss of different origin. Different types of topical formulations for administration of these compounds on the skin or mucosal surface are described, like shampoo, ointments, creams, gels, lotions, solutions, aerosols, etc., and for oral administration in the treatment of skin diseases. Also, cosmetic action of these compounds was described, in particular regenerating and smoothing of the skin.

Effects of 1-methylnicotinamide chloride (MNA^+) in some skin diseases were described in a recent publication (Gębicki J, Sysa-Jędrzejowska A, Adamus J, Woźniacka A, Rybak M, Zielonka J. 1-Methylnicotinamide: a potent anti-inflammatory agent of vita-

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min origin. *Pol J Pharmacol* 2003;55:109-112). It has been proposed that MNA^+ displays anti-inflammatory action, though the mechanism of this effect was not elucidated.

1-Methyl-3-acetylpyridinium salt (MAP^+), was described in a publication (Takashi Sakurai, Haruo Hosoya. Charge transfer complexes of nicotinamide-adenine dinucleotide analogs and flavine mononucleotide. *Biochim. Biophys. Acta* 1966;112(3):359-468).

Now it has been found that MAP^+ and some of the compounds described in WO00/40559, in particular MNA^+ and $MNAF^+$ possess unique pharmacological properties associated with their ability to release endogenous prostacyclin (PGI_2) from vascular endothelium, which property distinguishes them from closely structurally related nicotinamide, nicotinic acid, trigonelline and endogenous MNA^+ metabolites, such as 1-methyl-2-pyridone-5-carboxyamide (2-PYR) and 1-methyl-4-pyridone-3-carboxyamide (4-PYR). Accordingly, the above compounds, in particular MNA^+, can be effective in the prevention or the treatment of humans suffering from diseases associated with endothelial dysfunction, enhanced oxidative stress and insufficient endothelial prostacyclin (PGI_2) production or at enhanced risk of such diseases.

Description of Figures of the drawing

Fig.1. Scheme of the method for detection of thrombolytic action of drugs *in vivo* in rats according to Gryglewski.

Fig. 2. Thrombolytic response induced by intravenous administration of MNA^+ *in vivo* (30 mg/kg).

Fig. 3. Changes in plasma levels of 6-keto-$PGF_{1\alpha}$(•) and TXB_2 (◦) after intravenous administration of MNA^+ (30 mg/kg).

Fig. 4. Lack of significant thrombolytic response *in vivo* after intravenous administration of nicotinamide (30 mg/kg) or nicotinic acid (30 mg/kg).

Fig. 5 Lack of significant thrombolytic response *in vivo* after intravenous administration of 2-PYR (30 mg/kg) or trigonelline (30 mg/kg).

Fig. 6. Thrombolytic response induced *in vivo* by intravenous administration of MAP^+ (30 mg/kg).

Fig. 7. Changes in plasma levels of 6-keto-$PGF_{1\alpha}$(•) and TXB_2 (◦) after intravenous administration of MAP^+ in vivo (30 mg/kg).

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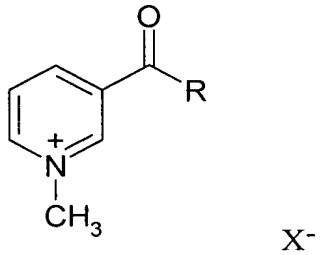
Fig. 8. Thrombolytic response induced by intravenous administration of $MNAF^+$ (30 mg/kg).

Fig. 9. Lack of effect of MNA^+ on collagen-induced (1 mg/ml)aggregation of platelets.

Fig. 10. Lack of effect of MNA^+ on latex-induced activation of neutrophils.

The present invention relates to the use of quaternary pyridinium salts of the formula I:



I

wherein R is NH_2, CH_3, or $N(H)CH_2OH$ group, and X a is pharmaceutically acceptable counterion,

for the preparation of a vasoprotective agent for the treatment and/or prevention of conditions or diseases associated with dysfunction of vascular endothelium, oxidative stress, and/or insufficient production of endothelial prostacyclin (PGI_2).

According to the present knowledge, a particularly preferred action of the compounds is their endothelial action associated with liberation of PGI_2, due to which these compounds can improve tissue perfusion, have antithrombotic, thrombolytic, antiapoptotic, antiatherosclerotic action, and protect gastric and gastrointestinal mucosa.

The advantage of the invention is that thrombolytic action of the compounds is not accompanied by hypotensive activity. Furthermore, this thrombolytic action is not linked with direct action on blood platelets. Also, these compounds do not act directly on leucocytes.

In one embodiment of the invention, said condition or disease is atherosclerosis (*atherothrombosis*) of a vascular bed of any kind, for ex. chronic coronary disease, ischemic cerebrovascular episode or artherosclerosis of the extremities.

In another embodiment of the invention said condition or disease is an acute cardiovascular event associated with atherosclerosis, in particular sudden cardiac death, acute

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coronary syndrome (including unstable coronary artery disease, myocardial infarct), the necessity of coronary angioplasty (PCI), coronary-aortal by-pass surgery (CABG), ischemic stroke, or necessity of peripheral circulation revascularization.

In yet another embodiment of the invention said condition or disease is selected from the group of risk factors for atherosclerosis (*atherothrombosis*), comprising the following: hypercholesterolemia, arterial hypertension, smoking, hyperhomocysteinaemia, insulin resistance, diabetes, menopause, aging, obesity, mental stress, infections, inflammatory states, including periodontal diseases, reumathoid arthritis, allograft vasculopathy or nitrate tolerance.

In yet another embodiment of the invention said condition or disease is thrombosis that is not related directly with atherosclerosis, in particular thrombosis associated with implantation of metallic vascular prostheses (stents), coronary-aortal by-pass surgery (CABG), hemodialysis, venous thrombo-embolic disease.

In a further embodiment of the invention said condition or disease associated with dysfunction of vascular endothelium is selected from the following group: chronic heart failure, pulmonary hypertension, microvascular diabetic complications, diabetic neuropathy, nephrotic syndrome, adults respiratory distress syndrome (ARDS), mucoviscidosis, asthma, chronic obstructive pulmonary disease (COPD), preeclampsia/eclampsia, erectile dysfunction, Stein-Leventhal syndrome, sleep apnea, systemic lupus erythematosus, sickle cell anemia, non-specific inflammatory bowel diseases, gastric or duodenal ulcers, glaucoma, primary amyloidosis, neurodegenerative diseases, in particular neurodegenerative disease selected from vascular dementia, Alzheimer's disease and Parkinson's disease.

Also advantageous is the use of the compounds according to the invention for the treatment and prophylaxis of gastric or duodenal ulcer.

As defined above, X- may be any physiologically acceptable counterion. Thus, salts of the formula I may be derived from any physiologically acceptable acid, both organic and inorganic. Suitable salts with inorganic acids are for example chloride, bromide, iodide and carbonate; suitable salts with organic acids may be salts with mono-, di- and triC_1-C_{18}carboxylic acids, for example acetate, benzoate, salicylate, hydroxyacetate, lactate, malonate and citrate. Preferred salts are chlorides, benzoates, salicylates, acetates, citrates and lactates; especially advantageous are chlorides.

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Specific compounds of the formula (I) are 1-methylnicotinamide (MNA^+) salts, 1-methyl-3-acetylpyridinium (MAP^+) salts and 1-methyl-N'-(hydroxymethyl)nicotinamide ($MNAF^+$) salts.

The invention in the second aspect provides a method of treatment and/or prevention of conditions or diseases associated with dysfunction of vascular endothelium, oxidative stress, and/or insufficient production of endothelial prostacyclin (PGI_2), in particular such as discussed above, comprising administration to a subject in a need of such treatment a therapeutically effective amount of a quaternary pyridinium salt of formula I as defined above.

Quaternary pyridinium salts of formula I may be administered in combination with other cardiovascular agent.

Pyridinium salts of formula I may be administered in particular orally, in the form of conventional oral preparations, such as tablets, capsules, oral solutions/suspensions in pharmaceutically acceptable liquid carrier, that may be prepared using conventional methods known in the art and include conventional pharmaceutical excipients and carriers.

Pyridinium salts of formula I may be in particular administered parenterally, in the form of injections, including subcutaneous and intravenous injections and infusions.

Other contemplated routes of administration of pyridinium salts of formula I are by inhalation, intranasally and rectally.

Daily dose of the pyridinium salts of the formula I may be in the range of 10 to 1000 mg and may be administered in single or divided doses.

In one embodiment of the method of treatment according to the invention said condition or the disease is atherosclerosis (*atherothrombosis*) of a vascular bed of any kind, for ex. chronic coronary disease, ischemic cerebrovascular episode or artherosclerosis of the extremities.

In another embodiment of the method of treatment according to the invention the condition or disease is an acute cardiovascular event associated with atherosclerosis, in particular sudden cardiac death, acute coronary syndrome (including unstable coronary artery disease, myocardial infarct), the necessity of coronary angioplasty (PCI), coronary-aortal by-pass surgery (CABG), ischemic stroke, or peripheral circulation revascularization.

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In yet another embodiment of the method of treatment according to the invention said condition or disease is selected from the group of risk factors for atherosclerosis (*atherothrombosis*), comprising the following: hypercholesterolemia, arterial hypertension, smoking, hyperhomocysteinaemia, insulin resistance, diabetes, menopause, aging, obesity, mental stress, infections, inflammatory states, including periodontal diseases, reumathoid arthritis, allograft vasculopathy or nitrate tolerance.

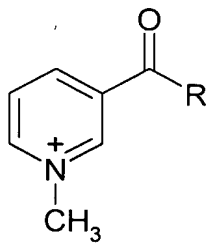
In yet another embodiment of the method of treatment according to the invention said condition or disease is thrombosis that is not related with atherosclerosis, in particular thrombosis associated with implantation of metallic and coated vascular prostheses (stents), coronary-aortal by-pass surgery(CABG), hemodialysis, venous thromboembolic disease.

In a further embodiment of the method of treatment according to the invention said condition or disease associated with dysfunction of vascular endothelium is selected from the following group: chronic heart failure, pulmonary hypertension, microvascular diabetic complications, diabetic neuropathy, nephrotic syndrome, chronic renal failure, adults respiratory distress syndrome (ARDS), mucoviscidosis, asthma, chronic obstructive pulmonary disease (COPD), preeclampsia/eclampsia, erectile dysfunction, Stein-Leventhal syndrome, sleep apnea, systemic lupus erythematosus, sickle cell anemia, non-specific inflammatory bowel diseases, gastric or duodenal ulcers, glaucoma, primary amyloidosis, neurodegenerative diseases, in particular neurodegenerative disease selected from vascular dementia, Alzheimer's disease and Parkinson's disease.

Also advantageous is the use of the compounds of formula I in the method of treatment and prophylaxis of gastric or duodenal ulcer.

The present invention relates also to a method for enhancing a prostacyclin levels in mammals, which comprises oral administration of an effective amount of a quaternary pyridinium salt of formula I as defined above.

The present invention provides also quaternary pyridinium salts of formula I



X-

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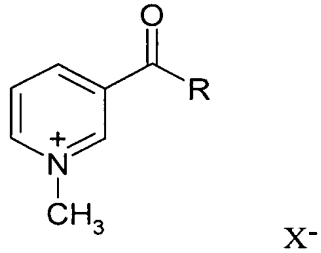
I

wherein R is NH_2, CH_3, or $N(H)CH_2OH$ group, and X is a counterion acceptable for consumption, for use in oral diet supplementation.

Pyridinium salts of formula I, when used as an oral diet supplement, enhance the prostacyclin level, thereby acting as vasoprotective agents.

The present invention provides further a use of pyridinium salts of the formula I



I

wherein R is NH_2, CH_3, or $N(H)CH_2OH$ group, and X is a counterion acceptable for consumption, for the preparation of a nutritional preparation for vascular protection in mammals in states or diseases associated with dysfunction of vascular endothelium, oxidative stress, and/or insufficient production of endothelial prostacyclin (PGI_2).

Said condition or disease wherein nutritional preparation may be administered is atherosclerosis, especially in patients with chronic coronary disease, ischemic cerebrovascular episode or artherosclerosis of the extremities.

Said condition or disease wherein the nutritional preparation may be administered may be also selected from the group comprising the following: hypercholesterolemia, arterial hypertension, smoking, hyperhomocysteinaemia, insulin resistance, diabetes, menopause, age-related insufficient production of endothelial prostacyclin, obesity, mental stress, infections, inflammatory states, including periodontal diseases, reumathoid arthritis, allograft vasculopathy or nitrate tolerance.

Said condition or disease wherein the nutritional preparation may be administered may be also thrombosis that is not related directly with atherosclerosis, in particular thrombosis associated with implantation of metallic vascular prostheses (stents), coronary-aortal by-pass surgery (CABG), hemodialysis, venous thrombo-embolic disease.

Specific pyridinium salts for use in diet supplementation and/or as nutritional preparation are compounds of the formula (I), wherein R is CH_3 group.

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Specific pyridinium salts for use in diet supplementation and/or as nutritional preparation are compounds of the formula (I), wherein R is NH_2 group.

Specific pyridinium salts for use in diet supplementation and/or as nutritional preparation are compounds of the formula (I), wherein R is $N(H)CH_2OH$ group.

Dietary supplements and nutritional preparations may have the form suitable for oral ingestion, such as tablets, capsules, solutions and suspensions for drinking, and similar, conventional and known in pharmaceutical art, and prepared according to techniques known in the art with the use of conventional excipients and carriers.

Advantageously, dietary supplement or nutritional preparation may incorporate at least 5% by weight of the pyridinium salt of the formula I.

Below Examples, which show pharmacological activity of pyridinium salts, are presented.

Thrombolytic activity was assessed using original method of Gryglewski et al., (Gryglewski RJ, Korbut R, Ocetkiewicz A, Stachura J. In vivo method for quantitation for anti-platelet potency of drugs. *Naunyn Schmiedebergs Arch Pharmacol* 1978;302:25-30), the scheme of which was shown on Fig 1.

Rats of body weight 300-350 g were anaesthetised (thiopental 95 mg kg^{-1} i.p.) and heparinised (heparin 800 i.u. kg^{-1} i.v.) and then cannulated. Cannules inserted in arteries were connected as follows: from left carotid artery to the pressure sensor; from left carotid artery through heated (37°C) line to the peristaltic pump that transfused arterial blood into extracorporeal circulation where it superfused (1.5 ml min^{-1}) a 3 cm long collagen strip hang on an auxotonic lever of an isotonic transducer (Harvard 386) provided with a spring damper. The collagen strip was cut from the Achilles tendon of a rabbit. After washing the strip blood returned through compensatory reservoir connected with left jugular vein to the circulation of an animal. During superfusion of the collagen strip a thrombi was built from blood platelets aggregates trapped in a fibrinogen network (visualized microscopically by Weigert method).

During the initial 20 - 30 min of superfusion the weight of the thrombi (instantly monitored) stabilized at the level of 70-100 mg and then stayed unchanged at this plateau to the end of the experiment, i.e. during next 3-5 hrs, unless an active medicament was injected intravenously to the animal. Thrombolytic response was detected by a fall in

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weight of a thrombi. Arterial blood pressure and thrombi weight were constantly recorded at the same time. Thus this model enabled the analysis of thrombolytic and hypotensive action of a compound (Fig. 1).

The analysis of the thrombolytic response in this experimental set-up was complemented by the assay of 6-keto-$PGF_{1\alpha}$, TXB_2 and PGE_2 levels in arterial blood plasma. For this purpose blood samples (500 µl) were collected into Eppendorff tubes with indomethacin and EDTA (final concentrations of 10 µM and 1 mM, respectively). Then, the blood samples were spun for 5 min at 2.000 x g and plasma samples were stored at -70°C before the assay. The prostanoids levels were assayed using commercial enzyme immunoassay ELISA kits (Cayman Chemical Co, Ann Arbor, MI).

Intravenous administration of MNA^+ (3 - 30 mg/kg) produced a concentration-dependent thrombolysis in Wistar rats with extracorporal circulation at the MNA^+ dose of 30 mg/kg. Single injection of MNA^+ at the dose of 30 mg/kg induced a long-lasting thrombolytic response at the level of 42 ± 4% at about 30 min after the injection and remained at approximately the same level for 2-3 hours of the observation period.. In contrast to MNA^+, nicotinamide, nicotinic acid, trigonelline and 2-PYR - endogenous metabolite of MNA^+ (both at 30 mg/kg) - failed to induce a significant thrombolytic response. Nicotinamide and nicotinic acid -induced responses were transient (less then 15-20 minutes) and at their maximum amounted merely to 9 ± 0.6 %, 5 ± 0.9 %, respectively). Trigonelline did not produce any thrombolytic response and response to 2-PYR was also very weak (<10%) and transient (shorter than 15 min). The potency and duration of thrombolytic responses to MNA^+, nicotinamide and nicotinic acid correlated with a pattern of 6-keto-$PGF_{1\alpha}$ release to arterial plasma induced by these compounds. Injection of MNA^+ (30 mg/kg) induced a substantial increase in levels of 6-keto-$PGF_{1\alpha}$ as early as 15 minutes after drug injection (from 104 ± 7 to 460 ± 58 pg/ml) which then reached its plateau (of around 400 pg/ml) for at least one hour. On the other hand neither TXB_2 nor PGE_2 levels changed significantly in response to MNA^+. Sluggish rise in TXB_2 levels was time-dependent and observed also after saline injection. Levels of 6-keto-$PGF_{1\alpha}$ did not increase after injection of nicotinamide or nicotinic acid (both compounds at 30 mg/kg).

In the presence of indomethacin (5 mg/kg) thrombolytic response to MNA^+ was abrogated, similarly as MNA^+-induced release of 6-keto-$PGF_{1\alpha}$. Importantly, thrombolytic response induced by MNA^+ (30 mg/kg) was not associated with a fall in arterial blood pressure. Collagen-induced aggregation in PRP in vitro was not affected by MNA^+ up to

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concentration of 10 mM, this excluding the possibility that thrombolytic action *in vivo* was due to the direct effect of MNA^+ on platelets. Furthermore MNA^+ (1 mM) did not inhibit latex-induced activation of neutrophils, this suggesting a possible selectivity of MNA^+ towards endothelium.

Fig. 2 shows thrombolytic response *in vivo* induced by MNA^+ (30 mg/kg), and Fig. 3 shows a concomitant increase in level of 6-keto-$PGF_{1\alpha}$ – a stable PGI_2 metabolite - in blood. Nicotinamide, nicotinic acid (Fig. 4), trigonelline and 2-PYR (Fig. 5) at the same or even higher dose displayed very weak thrombolytic activity. Both MAP^+ and $MNAF^+$ (30 mg/kg) (30 mg/kg) induced a thrombolytic response (Fig. 6 and Fig. 8) that was only slightly lower than that induced by 30 mg/kg of MNA^+. MAP^+ - induced thrombolysis was associated with the release of PGI_2 (Fig. 7), similarly as in the case of MNA^+-induced thrombolysis. MNA^+ (30-300 mg/kg) did not cause hypotension. As shown on Fig. 9 and Fig. 10, MNA^+ lacked direct action on aggregation of platelets and activation of neutrophils. Aggregation of platelets depends on COX1-TXA_2 and was abrogated by aspirin, whereas latex-induced chemiluminescence of neutrophils depends on NADPH oxidase and was abrogated by DPI and apocynin.

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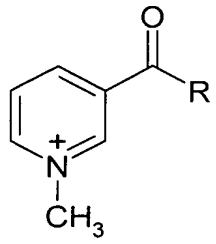
Claims

1. A use of quaternary pyridinium salts of the formula I:



X^-

I

wherein R is NH_2, CH_3, or $N(H)CH_2OH$ group, and X is a pharmaceutically acceptable counterion,

for the preparation of a vasoprotective agent for the treatment and/or prevention of conditions or diseases associated with dysfunction of vascular endothelium, oxidative stress, and insufficient production of endothelial prostacyclin (PGI_2).

2. The use in accordance with claim 1, wherein said condition or disease is atherosclerosis.

3. The use in accordance with claim 1, wherein said condition or disease is an acute cardiovascular event associated with atherosclerosis, in particular sudden cardiac death, acute coronary syndrome (including unstable coronary artery disease, and myocardial infarct), the necessity of coronary angioplasty (PCI), coronary-aortal by-pass surgery (CABG), ischemic stroke, or necessity of peripheral circulation revascularization.

4. The use in accordance with claim 1, wherein said condition or disease is atherosclerosis in patients with chronic coronary disease, ischemic cerebrovascular episode or artherosclerosis of the extremities.

5. The use in accordance with claim 1, wherein said condition or disease is selected from the group of risk factors for atherosclerosis (*atherothrombosis*), comprising the following: hypercholesterolemia, arterial hypertension, smoking, hyperhomocysteinaemia, insulin resistance, diabetes, menopause, age-related impairement of prostacycline

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synthesis, obesity, mental stress, infections, inflammatory states, including periodontal diseases, reumathoid arthritis, allograft vasculopathy and nitrate tolerance.

6. The use in accordance with claim 1, wherein said condition or disease is thrombosis that is not related with atherosclerosis, in particular thrombosis associated with implantation of metallic vascular prostheses (stents), coronary-aortal by-pass surgery(CABG), hemodialysis, venous thrombo-embolic disease.

7. The use in accordance with claim 1, wherein said condition or disease associated with dysfunction of vascular endothelium is selected from the following group: chronic heart failure, pulmonary hypertension, microvascular diabetic complications, diabetic neuropathy, nephrotic syndrome, chronic renal failure, adults respiratory distress syndrome (ARDS), mucoviscidosis, asthma, chronic obstructive pulmonary disease (COPD), preeclampsia/eclampsia, erectile dysfunction, Stein-Leventhal syndrome, sleep apnea, systemic lupus erythematosus, sickle cell anemia, non-specific inflammatory bowel diseases, gastric or duodenal ulcers, glaucoma, primary amyloidosis, and neurodegenerative diseases.

8. The use in accordance with claim 7, wherein said neurodegenerative disease is selected from vascular dementia, Alzheimer's disease and Parkinson's disease.

9. The use in accordance with claim 7, wherein said disease is gastric or duodenal ulcer.

10. The use in accordance with any one of claims 1 to 9, wherein said vasoprotective agent is in a form for oral administration.

11. The use in accordance with any one of claims 1 to 9, wherein said vasoprotective agent is in a form for parenteral administration.

12. The use in accordance with any one of claims 1 to 11, wherein R is CH_3 group.

13. The use in accordance with any one of claims 1 to 11, wherein R is NH_2 group.

14. The use in accordance with any one of claims 1 to 11, wherein R is $N(H)CH_2OH$ group.

15. A method for treatment and/or prevention of conditions or diseases associated with dysfunction of vascular endothelium, oxidative stress and insufficient production of endothelial PGI_2, comprising administration to a subject in a need thereof a therapeutically effective amount of a quaternary pyridinium salt of formula I:

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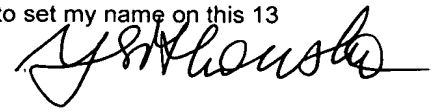

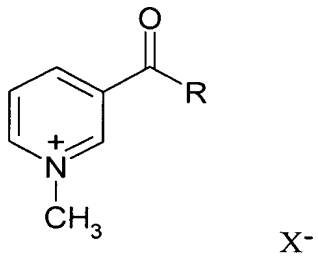

I

wherein R is NH_2, CH_3, or $N(H)CH_2OH$ group, and X is a pharmaceutically acceptable counterion.

16. The method in accordance with claim 15, wherein said condition or disease is atherosclerosis.

17. The method in accordance with claim 15, wherein said condition or disease is an acute cardiovascular event associated with atherosclerosis, in particular sudden cardiac death, acute coronary syndrome (including unstable coronary artery disease, myocardial infarct), the necessity of coronary angioplasty (PCI), coronary-aortal by-pass surgery (CABG), ischemic stroke, or peripheral circulation revascularization.

18. The method in accordance with claim 15, wherein said condition or disease is atherosclerosis in patients with chronic coronary disease, ischemic cerebrovascular episode or artherosclerosis of the extremities.

19. The method in accordance with claim 15, wherein said condition or disease is selected from the group of risk factors for atherosclerosis (*atherothrombosis*), comprising the following: hypercholesterolemia, arterial hypertension, smoking, hyperhomocysteinaemia, insulin resistance, diabetes, menopause, age-related impairement of prostacycline synthesis, obesity, mental stress, infection, inflammatory states, including periodontal diseases, reumathoid arthritis, allograft vasculopathy, nitrate tolerance.

20. The method in accordance with claim 15, wherein condition or disease is thrombosis that is not related directly with atherosclerosis, in particular thrombosis associated with implantation of metallic vascular prostheses (stents), coronary-aortal by-pass surgery(CABG), hemodialysis, venous thrombo-embolic disease.

21. The method in accordance with claim 15, wherein said condition or disease is selected from the following group: chronic cardiac failure, pulmonary hypertension, microvascular diabetic complications, diabetic neuropathy, nephrotic syndrome, chronic renal failure, adults respiratory distress syndrome (ARDS), mucoviscidosis, asthma,

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chronic obstructive pulmonary disease (COPD), preeclampsia/eclampsia, erectile dysfunction, Stein-Leventhal syndrome, sleep apnea, systemic lupus erythematosus, sickle cell anemia, non-specific inflammatory bowel diseases, gastric or duodenal ulcers, glaucoma, primary amyloidosis, neurodegenerative diseases.

22. The method in accordance with claim 21, wherein said neurodegenerative disease is selected from vascular dementia, Alzheimer's disease and Parkinson's disease.

23. The method in accordance with claim 3021 wherein said disease is gastric or duodenal ulcer.

24. The method in accordance with claim 15, wherein pyridinium derivative is administered orally.

25. The method in accordance with claim 15, wherein pyridinium derivative is administered parenterally.

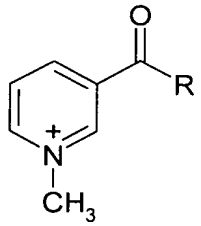
26. The method in accordance with claim 15, wherein R is CH_3 group.

27. The method in accordance with claim 15, wherein R is NH_2 group.

28. The method in accordance with claim 29, wherein R is $N(H)CH_2OH$ group.

29. The method in accordance with claim 15, wherein pyridinium salt is administered together with other cardiovascular agent.

30. A method for enhancing a prostacyclin levels in mammals, which comprises oral administration of an effective amount of a quaternary pyridinium salt of formula I:



X^-

I

wherein R is NH_2, CH_3, or $N(H)CH_2OH$ group, and X is a pharmaceutically acceptable counterion.

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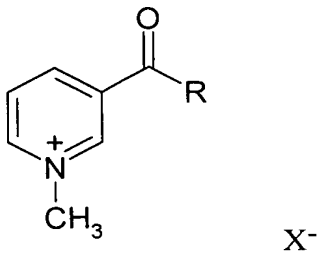
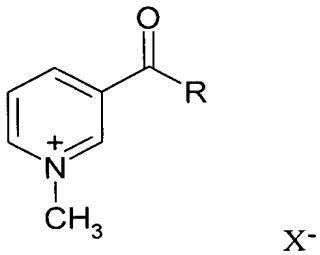
31. Quaternary pyridinium salts of formula I:



I

wherein R is NH_2, CH_3, or $N(H)CH_2OH$ group, and X is a counterion acceptable for consumption, for use in oral diet supplementation.

32. A use of quaternary pyridinium salts of formula I:



I

wherein R is NH_2, CH_3, or $N(H)CH_2OH$ group, and X is a counterion acceptable for consumption, for the preparation of a nutritional preparation for vascular protection in mammals in states or diseases associated with dysfunction of vascular endothelium, oxidative stress, and/or insufficient production of endothelial prostacyclin (PGI_2)

33. The use in accordance with claim 32, wherein said condition or disease is atherosclerosis, in particular in patients with chronic coronary disease, ischemic cerebrovascular episode or artherosclerosis of the extremities.

34. The use in accordance with claim 32, wherein said condition or disease is selected from the group of risk factors for atherosclerosis (*atherothrombosis*), comprising the following: hypercholesterolemia, arterial hypertension, smoking, hyperhomocysteinaemia, insulin resistance, diabetes, menopause, aging, obesity, mental stress, infections, inflammatory states, including periodontal diseases, reumathoid arthritis, allograft vasculopathy or nitrate tolerance.

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35. The use in accordance with claim 32, wherein condition or disease is thrombosis that is not related with atherosclerosis, in particular thrombosis associated with implantation of metallic and coated vascular prostheses (stents), coronary-aortal by-pass surgery(CABG), hemodialysis, venous thrombo-embolic disease.

36. The use in accordance with claim 32, wherein insufficient production of endothelial prostacyclin is age-related.

36. The use in accordance with claims 32 to 35, wherein R is CH_3 group.

37. The use in accordance with claims 32 to 35, wherein R is NH_2 group.

38. The use in accordance with claims 32 to 35, wherein R is $N(H)CH_2OH$ group.

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Fig.1 Scheme of the method for detection of thrombolytic action of drugs *in vivo* in rats (according to Gryglewski et al..)

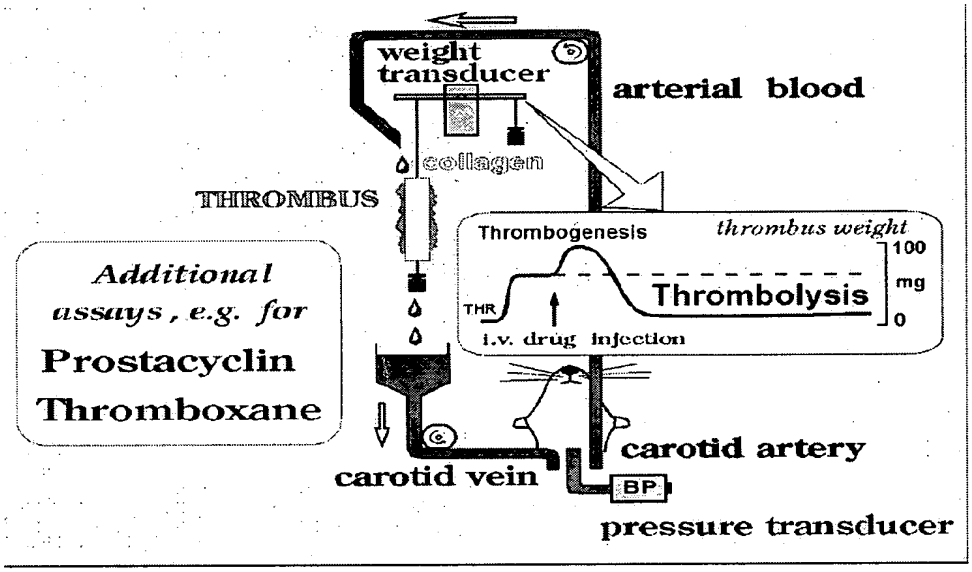

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Fig. 2. Thrombolytic response *in vivo* induced by intravenous MNA+ (30 mg/kg) administration

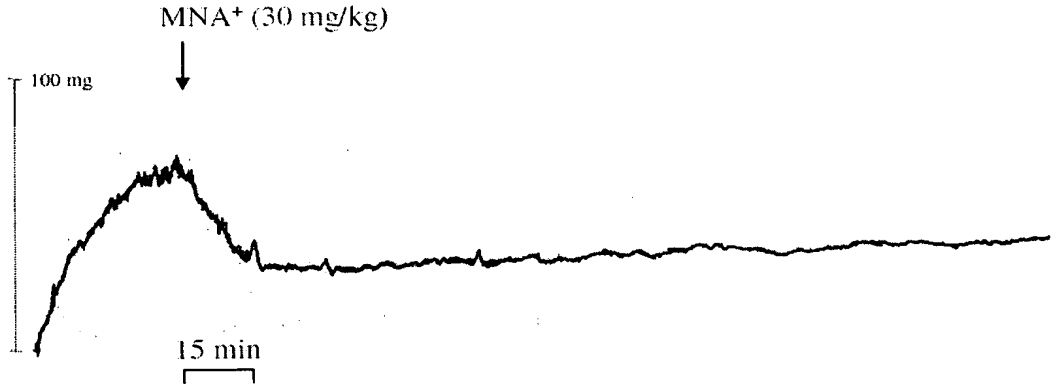

Fig. 3. Changes in plasma levels of 6-keto-$PGF_{1\alpha}$ (•) and TXB_2 (◦) after intravenous administration of MNA+ (30 mg/kg)

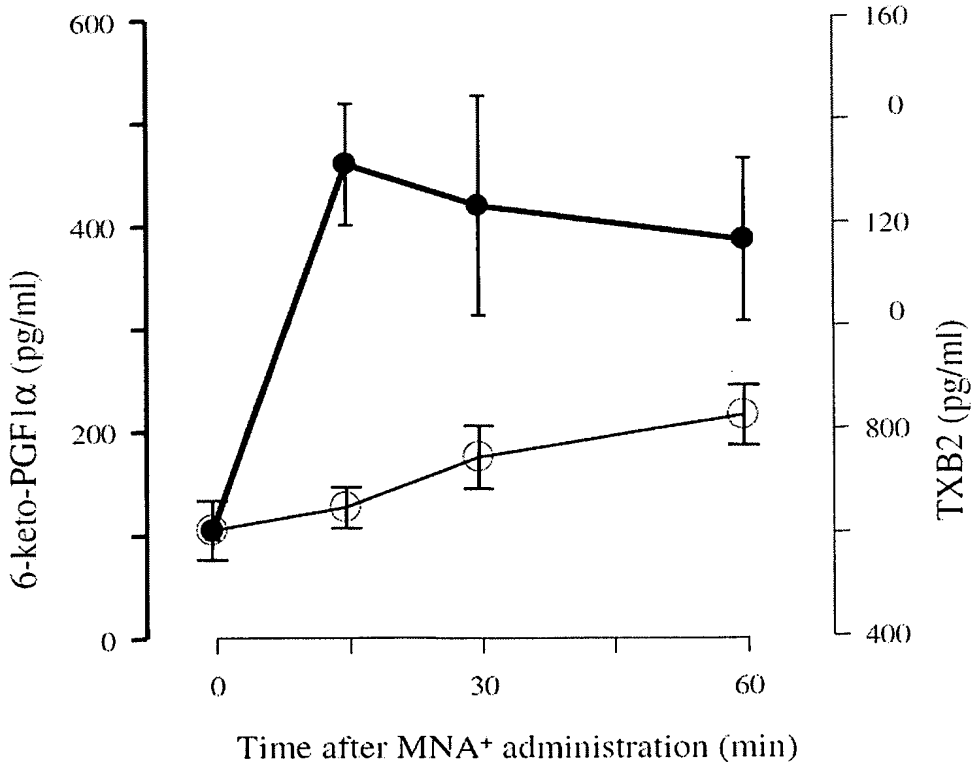

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Fig. 4. Lack of thrombolytic response *in vivo* after intravenous administration of nicotinamide (30 mg/kg) or nicotinic acid (30 mg/kg).

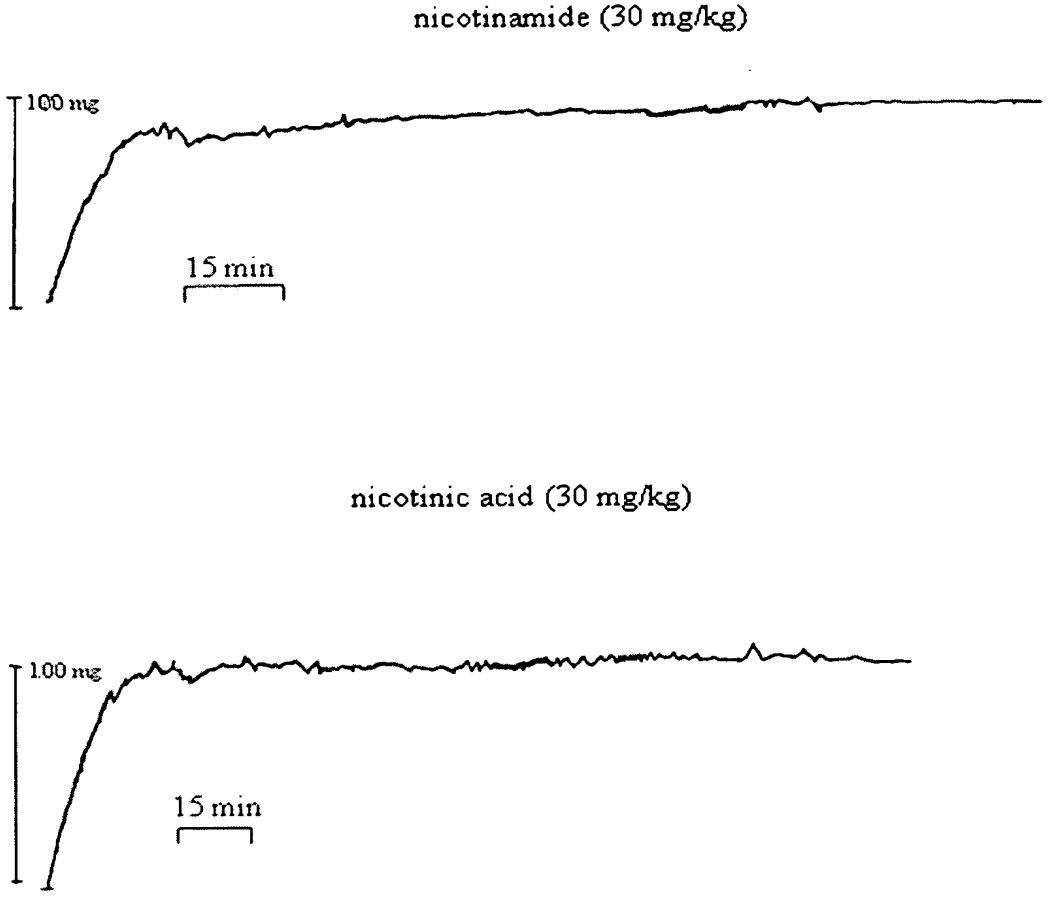

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Fig. 5. Lack of thrombolytic response *in vivo* after intravenous administration of 2-PYR (30 mg/kg) or trigonelline (30 mg/kg).

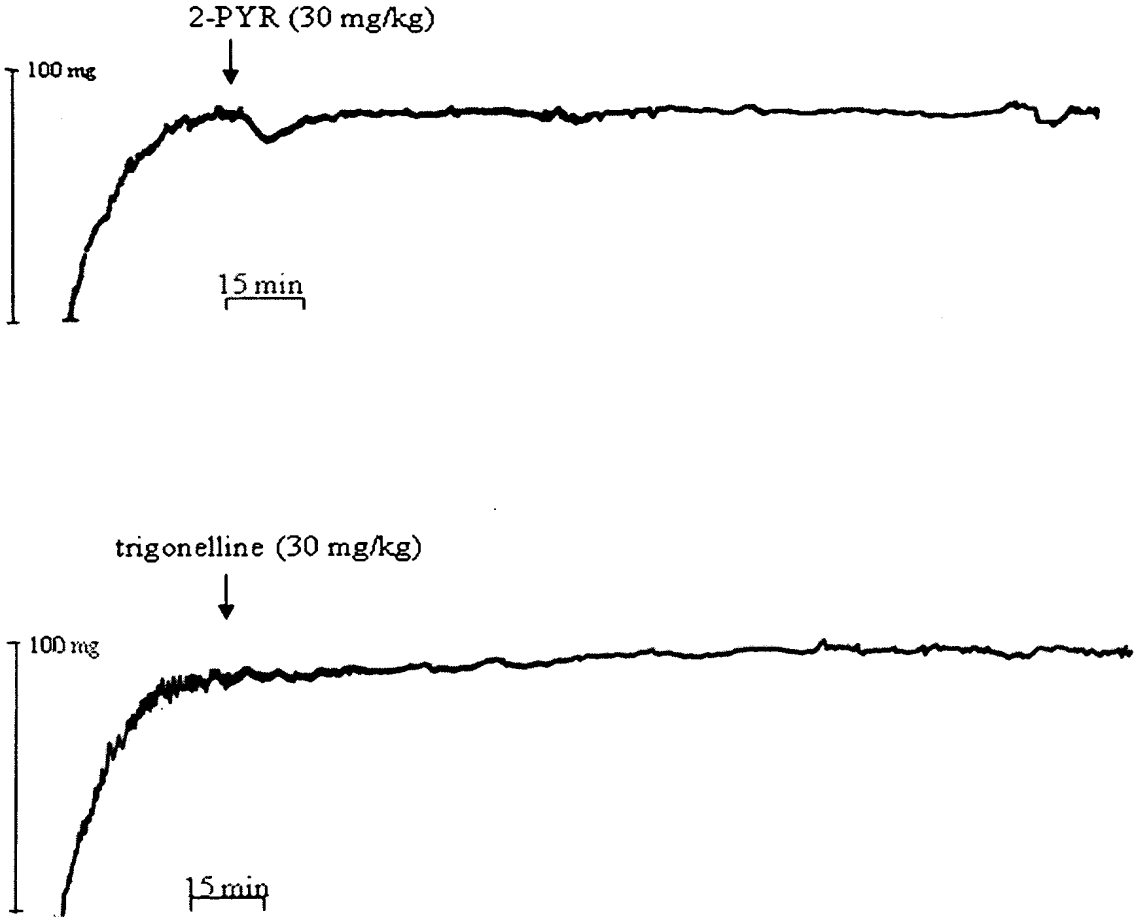

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Fig. 6. Thrombolytic response *in vivo* induced by intravenous administration of MAP^+ (30 mg/kg)

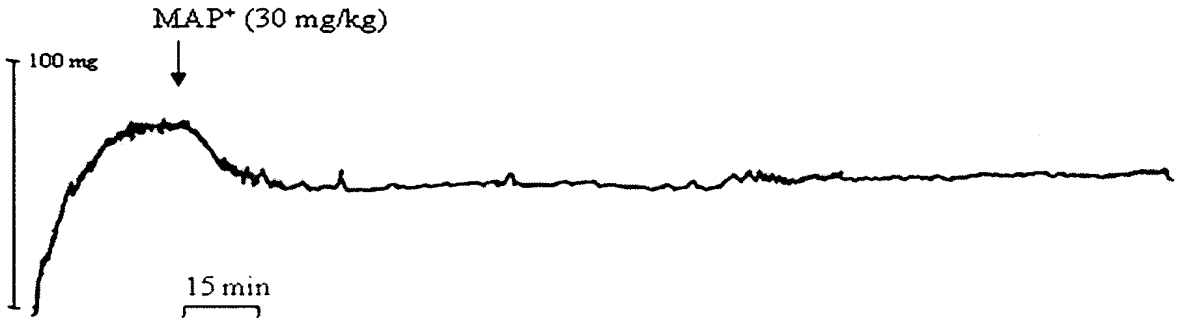

Fig. 7. Changes in plasma levels of 6-keto-PGF_{1a} (•) and TXB_2 (◦) after intravenous administration of MAP^+ (30 mg/kg)

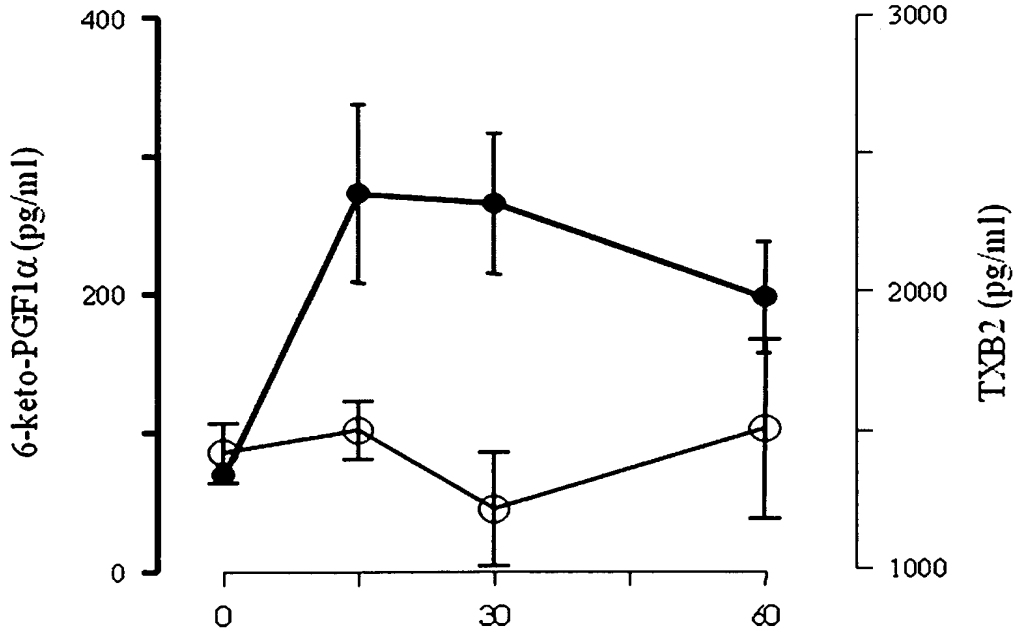

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Fig. 8. Thrombolytic response *in vivo* induced by intravenous administration of $MNAF^+$ (30 mg/kg)

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Fig. 9 Lack of effect of MNA^+ on collagen-induced aggregation of platelets (1 μg/ml)

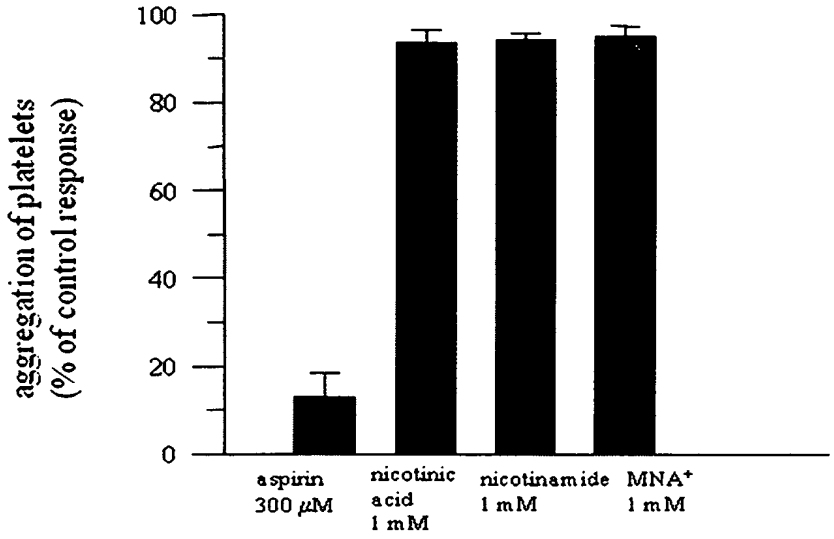
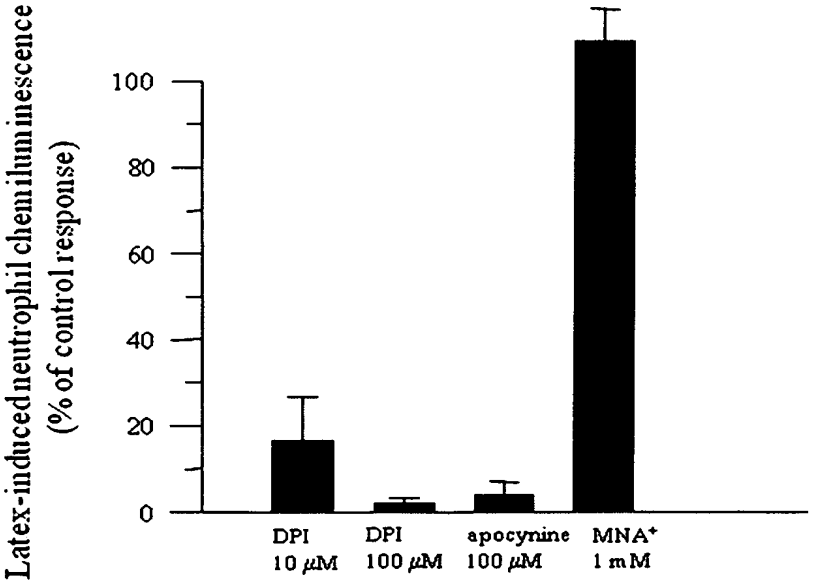


Fig. 10 Lack of effect of MNA^+ on latex-induced activation of neutrophils



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